HEAT-SENSITIVE MEDIUM FOR SEPARATING SPECIES IN A SEPARATING CHANNEL

The present invention relates to the field for separating, identifying and/or analyzing particles, molecules or macromolecules, and more particularly nucleic acids, in a channel, for example in a microfluidic system or more particularly in the context of capillary electrophoresis.

Gel electrophoresis has numerous applications for separating charged particles, molecules and macromolecules, and in particular biological macromolecules such as nucleic acids (DNA, RNA, oligonucleotides), proteins, polypeptides, glyco-peptides and polysaccharides. A particularly important application is sequencing, that is to say the reading of the genetic code of DNA. It is most often carried out in flat and macroscopic gels (having a thickness of the order of 1 to several mm), composed of agarose or polyacrylamide. More recently, other gels derived from acrylamide and a whole series of acrylate or methacrylate gels have been proposed for improving this or that property of the gel. In particular, in patent US 5,164,057, there has been proposed the use of polymers having a lower critical solution temperature (abbreviated hereinafter LCST in the present application) for controlling the hydrophilic/hydro-phobic character in a permanently and irreversibly crosslinked gel, used in electrophoresis.

In spite of these improvements, planar gel electro-phoresis has many disadvantages.

It requires a relatively substantial amount of manual work for the preparation of the gel, which can only be used once, and for subsequent visualization of the migration distances. Reproducibility of the experiments from one gel to another is difficult to obtain because

the properties of the gel depend on the exact preparation conditions. Given the production of heat linked to the current, fairly low low voltages have to be applied, leading to long separation times. Finally, planar electrophoresis is difficult to automate and to quantify. For these different reasons, macroscopic gel electrophoresis tends to be overtaken by techniques in which the separation or more generally the analysis of the constituents of a sample is carried out in channels with a high surface/volume ratio, such as cylindrical capillaries, or channels with a planar submillimetric section which are prepared in a material (which are called "chips", microchannels or microfluidic systems). Examples of such separations are given for example in "Capillary electrophoresis in analytical biotechnology", Righetti ed., CRC press, 1996, or in Cheng, J. et al., (1996), Molecular Diagnosis, 1, 183-200.

In the text which follows, all the methods involving an electrophoretic separation in one channel or several channels of which at least one of the dimensions is of a submillimetric dimension will be designated by the name "capillary electrophoresis" (CE). "Microfluidic system" will designate more generally any system in which the analysis of species is carried out by virtue of the transport of the said species and/or of fluids within a channel or a set of channels of which at least one of the dimensions is submillimetric.

CE and microfluidic systems allow more rapid and higher resolution separations than gels, do not require an anticonvective medium, and their properties have been widely used to carry out separations of ions in a liquid medium.

However, a large proportion of analytes which it is desired to separate by electrophoresis, and in particular those encountered in biology, have, in a

homogeneous viscous medium, a mobility which is independent of their size and can only be satisfactorily separated in a medium with obstacles. The gels used in traditional electrophoresis in particular consist of portions of macromolecular chains or of fibres which are connected to each other and cannot be crossed by the analytes. The latter have to create passages along the pores present between the macromolecular segments of the gel, which gives rise to the desired separation. In the first applications of CE to DNA and to proteins, use was often made of permanent gels which are crosslinked inside the capillary. This has many disadvantages: the preparation of a truly homogeneous gel with no bubbles inside a capillary is delicate. The gel becomes degraded relatively rapidly by hydrolysis and is "contaminated" by impurities present in the samples, which eventually damage the performance features of the separation, or even block the capillary after a limited number of separations. Given the production cost and/or the difficulty of manufacturing a capillary filled with gel, this defect makes the cost of this approach prohibitive.

Currently, the great majority of separations of biological macromolecules carried out in CE make use of solutions of entangled linear water-soluble polymers having the advantage of being replaceable as often as necessary. At a fairly high concentration, that is significantly above the entanglement threshold, the various polymers in the solution become entangled and constitute a continuous transient network of topological obstacles which cannot be crossed by the analytes, thus conferring electrophoretic separation properties on the solution.

These water-soluble polymer solutions are satisfactory in some applications, but also have numerous limitations.